Lab 4: Parasite Aggregation + Host Immune Response

Today's Lab

1. Go over some basics about innate immunity in insects
2. Calculate Mite Burdens
3. Melanization Assay
4. Dissection and Calculation of Nematode Burden
5. Data Analysis Tutorial

Horned Passalus Beetle (*Odontotaenius disjunctus*)

Family Passalidae
- “Bessbugs” or “Leather beetles
- Nearly 500 species, but only two species native to the US! (More common in the new world tropics)

Horned Passalus
- Large (1.5-2 inches as adults)
- Small golden hairs line middle legs, pronotum, and antennae.

Horned Passalus Beetle (*Odontotaenius disjunctus*)

Make large “galleries” in fallen, decaying hardwoods. Form subsocial groups, where adults will tend to larve and eggs of others

Generally use a two-stage digestion process-they first chew and digest wood and expel as powdery frass. After frass is colonized and further decomposed by fungi and bacteria, they consume it again (similar to termites)

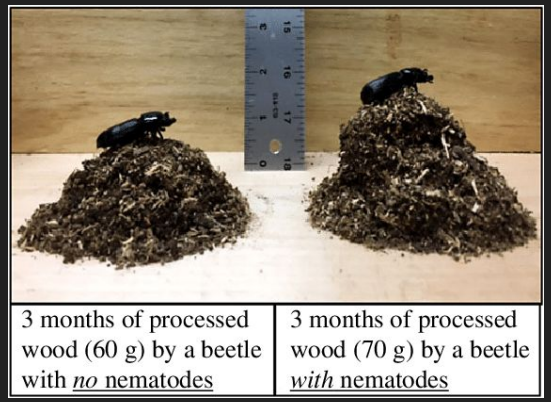
Parasites

Nematode parasite: *Chondronema passali*

Variable intensity of infection (some 0-5, others 100+)

Alter feeding rate

Potential behavioral effects



3 months of processed wood (60 g) by a beetle with *no* nematodes	3 months of processed wood (70 g) by a beetle *with* nematodes

The New York Times

TRILOBITES

Parasites Infect These Beetles. It Might Be a Good Thing.

Parasites

Also have a number of mites species

Some commensal (feeding on things in wood alongside the beetle)

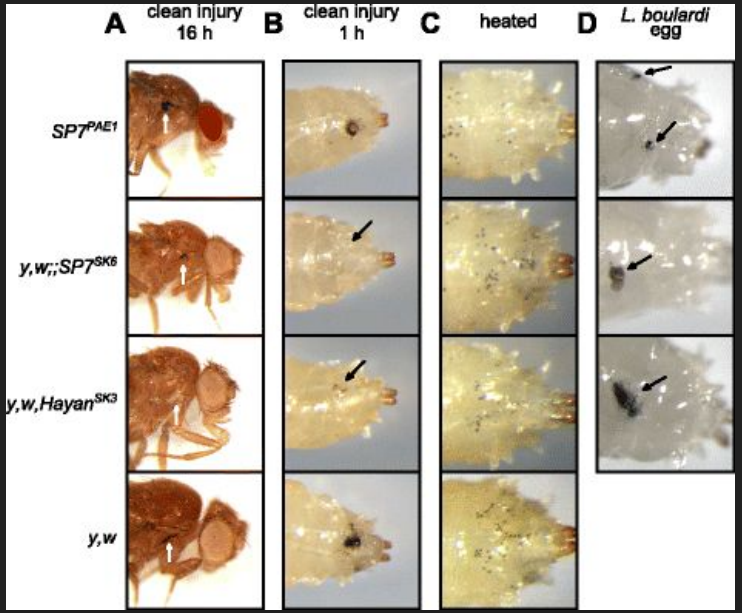
Some parasitic (sucking hemolymph)

Insect Immunity

Insects have to solely rely on their innate immunity!

The melanization response is triggered through a number of pathways, including in response to wounding, or recognition of foreign pathogens.

Melanization serves a number of roles, including hardening of clots, as well as being toxic to macroparasites, fungi, bacteria, and even viruses (Rodriguez-Andres et al., 2012).



Melanization Assay:

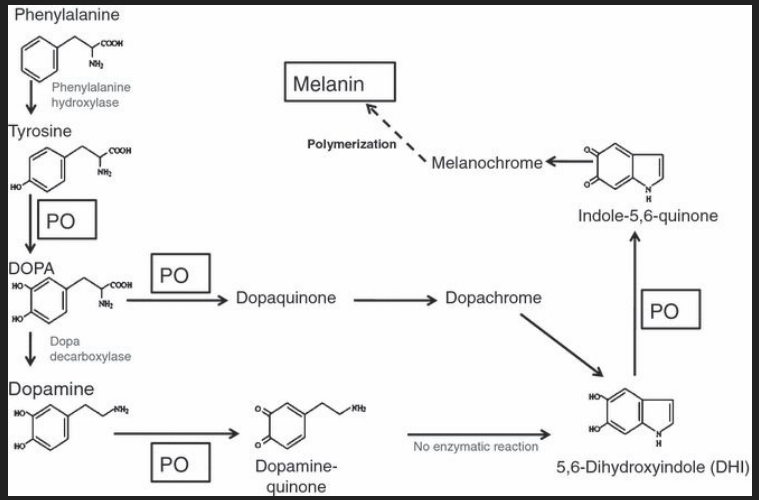
Rate limiting step of melanogenesis: Synthesis of melanin itself from precursor products

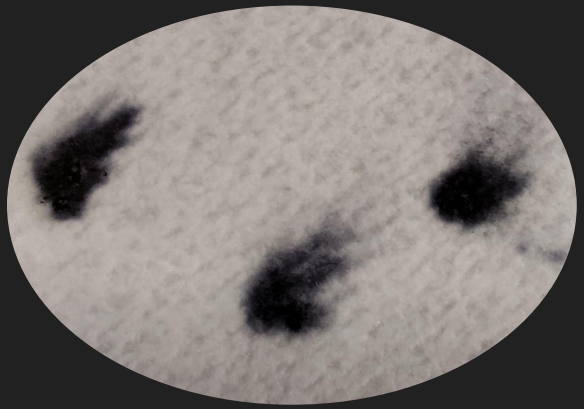
Catalyzed by **Phenol Oxidase**

Our assay: Take hemolymph samples, and place them in a solution with lots of molecular precursor to melanin (L-3, 4-dihydroxyphenylalanine, or (**L-DOPA**)

We asses phenol oxidase activity by characterizing darkness of resulting melanin splotch

(Methods adapted from Nelson et al. 2002)





Research Question:

Is the strength of the melanization response in *Odontotaenius disjunctus* related to…

1) Ectoparasitic Mite Burden?
2) Endoparasitic Nematode Burden?

Sources

Rodriguez-Andres, J., Rani, S., Varjak, M., Chase-Topping, M. E., Beck, M. H., Ferguson, M. C., ... & Kohl, A. (2012). Phenoloxidase activity acts as a mosquito innate immune response against infection with Semliki Forest virus. PLoS pathogens, 8(11), e1002977.

Sorrentino, R. P., Small, C. N., & Govind, S. (2002). Quantitative analysis of phenol oxidase activity in insect hemolymph. Biotechniques, 32(4), 815-823.

González-Santoyo, I., & Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the insect immune system. Entomologia Experimentalis et Applicata, 142(1), 1-16.